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Study on the Volatile Compounds of Fish Sauces—Shottsuru, Nampla and Noucman

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Comparative study on the aromas of three kinds of fish sauces, Shottsuru, Nampla and Noucmam was conducted. Volatile compounds of all samples were separately collected by steam distillation, and separated and identified by gas chromatography and gas chromatography mass-spectrometry, respectively.

Subjectively, these three fish sauces revealed aromas different from each other. A total of 50, 44 and 49 volatile compounds were identified in Shottsuru, Nampla and Noucmam respectively. Acids, alcohols, nitrogen-containing compounds, sulfur-containing compounds, lactones, esters, phenols, carbonyls and hydrocarbons were among the main groups of volatile compounds identified. The differences in the aromas of the samples were thought to be due to the differences in the level of concentrations of the major acids. Moreover, some differences in the kinds of the minor volatile compounds were possible contributing factors in the differences of the total aromas.

Fish sauce is a clear brown liquid traditionally produced and widely used in Southeast Asia and to some extent in some parts of the world. Since the staple food of the Southeast Asian people is rice which is rather flat in taste, fish sauce is commonly used as a condiment to give taste to its flatness. It also provides a supplement to some for the protein requirements of the people.

Fish sauces come in different names such as Noucmam in Vietnam, Nampla in Thailand, Budu in Malaysia, Ketjap in Indonesia, Ngapi in Burma and Patis in the Philippines. In Japan, the product is called Shottsuru,[1] which has been used for the same purpose as soy sauce and is prepared from sardines, cuttle fish, herring or fish waste materials.

The aroma of fish sauce has been studied by several workers.[2~8] Three major notes were reported to comprise the aroma of fermented fish sauces,[5] a cheesy note mainly due to low fatty acids, an ammoniacal note due to ammonia and amines, a meaty note which may be due to other compounds. The latter was supposed to be complicated but it was believed to be produced in mature sauces.[5]

Although different countries use different species of fishes and varied methods of producing fish sauces, the basic principle in the production is almost the same and it is thought with interest to compare the characteristic aroma of some of the commonly used fish sauces.

McIver *et al.*[6] reported a number of volatile compounds in Nampla and Nonaka *et al.*[7] also reported some volatile compounds in their study on Shottsuru and Noucmam. In our study on the aroma of Patis,[3] we also reported various aroma compounds. Comparing the results of these reports, a few similarities as well as differences were noticed in the findings. However, since the methods used in the above analyses varied from the collection of volatile compounds to the columns of the gas chromatographic conditions, it would be hard to compare the results of these studies. In order to be able to compare reliably the aromas of fish sauces, three kinds of sauces were analyzed using the same procedure used in the study on the aroma of Patis.[3]

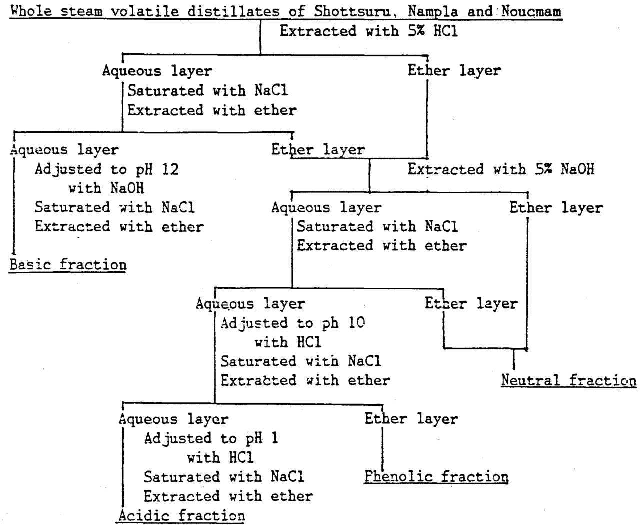

FIG. 1. Fractionation of the Whole Steam Volatile Distillates of Shottsuru, Nampla and Noucman.

MATERIALS AND METHODS

Commonly used fish sauces such as Shottsuru, produced by the Kakizaki Company in Akita Prefecture, Japan and commercially obtained was used. Noucmam, a product of Phu Quoc province in Vietnam and Nampla which was produced in Thailand and commercially purchased in Japan were also used. All these samples were obtained and analyzed in 1984.

Collection of volatiles. All these samples with a volume of 1,000 ml each were steam-distilled under reduced pressure at a temperature maintained at about 40∼45°C for about 4 hr. Each distillate of about 700 ml were extracted with about 400 ml of ethyl ether and the extracts were added with anhydrous sodium sulfate and allowed to stand overnight to ensure complete removal of water. Each was then concentrated in the usual manner at the temperature of about 30∼35°C to about 1 ml where an ethereal smell could hardly be felt. For a convenient and easy interpretation of subsequent analysis, a preliminary separation of the distillates were carried out by fractionating them into four fractions, neutral, basic, acidic and phenolic, using the procedure explained in Fig. 1. All these fractions were separately concentrated using the same procedure mentioned above.

Gas chromatography (GC). For the separation of the flavor compounds, each fraction was separately analyzed with a Hitachi 663-30 Model gas chromatograph equipped with a flame ionization detector (FID). A fused silica column (0.25 mm i.d. × 25 m) coated with PEG 20M was used. The column temperature was programmed from 60 to 170°C at the rate of 4°C/min. The injection port and detector were kept at 180 and 230°C, respectively. Nitrogen was used as a carrier gas with a flow rate of about 1.0 ml/min at a split ratio of 1 : 40.

Gas chromatography-mass spectrometry (GC-MS). After analysis by GC, each sample was analyzed by GC-MS for identification of volatile compounds. A Hewlett Packard 5790 Model gas chromatograph with a fused silica column (0.25 mm i.d. × 50 m) coated with Carbowax 20M was used. The column temperature was programmed from 60 to 170°C at the rate of 4°C/min, the injection port at 180°C and the detector (FID) at 230°C. The carrier gas was helium with a flow rate of about 1.0 ml/min and a split ratio of 1 : 28. A mass spectrometer JEOL JMS-DX 300 Model attached to the Hewlett Packard 5790 GC was used. Analysis was conducted using the electron impact ionization method at 70 eV. The Interface temperature was maintained at 200°C.

Identification of aroma compounds. Aroma compounds were identified by comparing their mass spectra with the published authentic spectra.[9] Authentic samples of compounds identified by mass spectrometry were used to check retention times using gas chromatography.

RESULTS AND DISCUSSION

In our study on the aroma of Patis,[3] 66 volatile compounds of various groups were identified and 16 of these were volatile acids, the largest group of volatile compounds identified. Five major acids were found in the acidic fraction, in which *n*-butanoic acid was

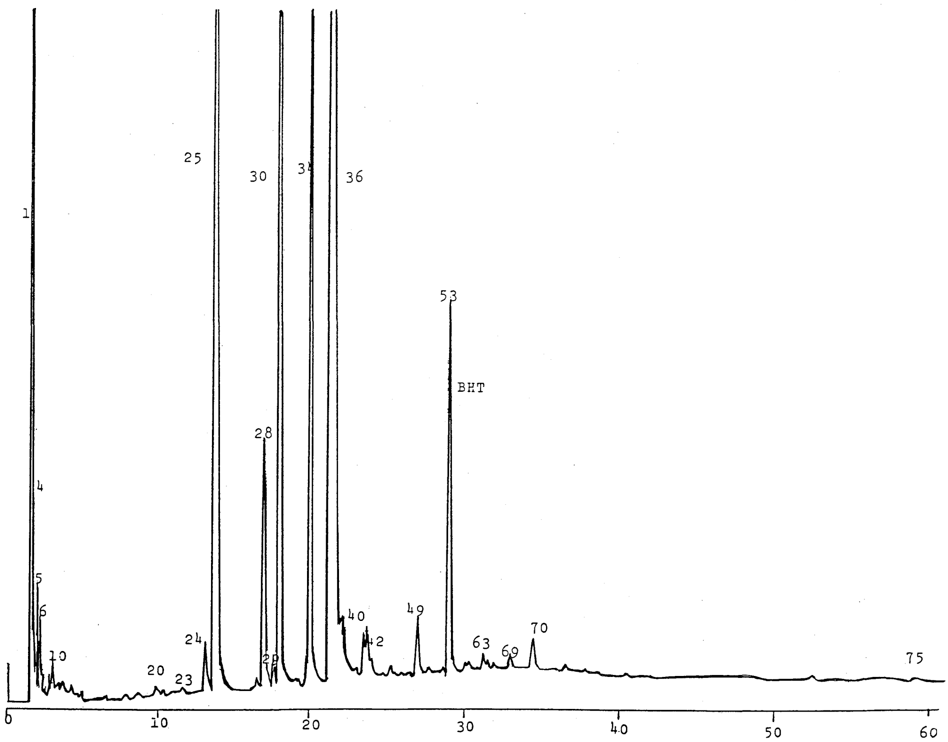

FIG. 2. Gas Chromatogram of the Whole Volatile Distillate of Shottsuru.

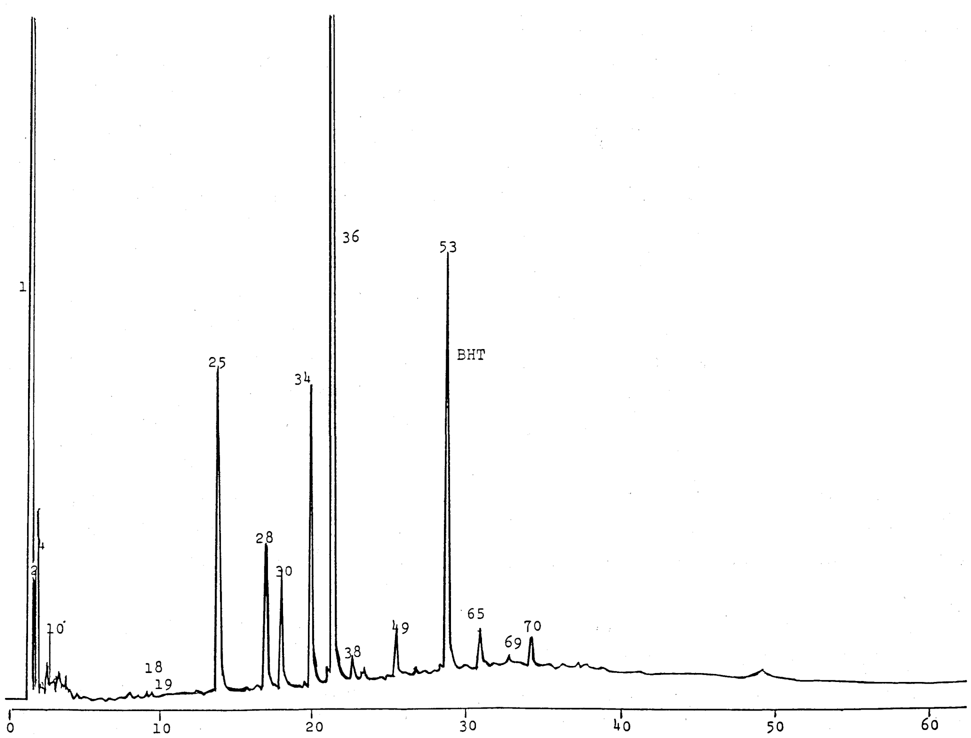

FIG. 3. Gas Chromatogram of the Whole Volatile Distillate of Nampla.

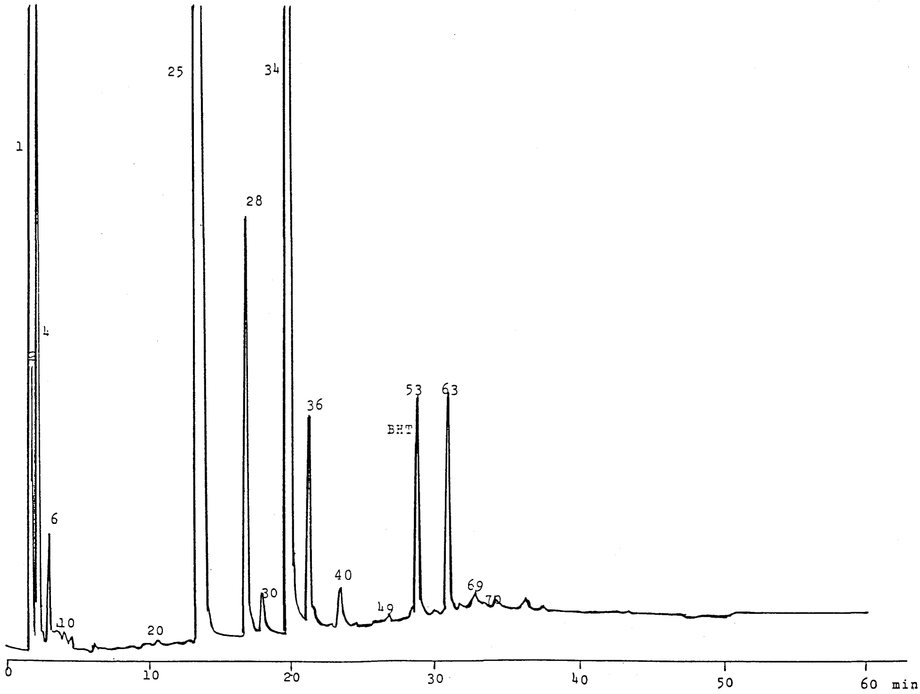

FIG. 4. Gas Chromatogram of the Whole Volatile Distillate of Noucmam.

the most abundant.[3)]

Subjectively, Shottsuru is a little fishy, cheesy and rancid with a sweet and a little burnt odor. It has a somewhat stimulating smell but to a lesser degree compared with Patis.[3)] A very faint soy sauce like smell could be felt in this sauce. Nampla has a more stimulating smell than Shottsuru, more fishy and is also cheesy and rancid. It is a little sweet and very slightly burnt. Noucmam exhibited a considerably burnt smell typical that of smoked products, particularly that of fish. Like the other fish sauces analyzed, it also has a cheesy and rancid odor, fishy and a little sweet. Although these fish sauces possess some similarities in their aroma they differ somehow to some degree in intensity which implies that the whole aroma of fish sauces differ in characteristics in some aspects.

In this study, volatile compounds considered to be responsible for the aroma of Shottsuru, Nampla and Noucmam were investigated and compared. Steam distillates of these samples were analyzed with a gas chromatography and their chromatograms are shown in Figs. 2, 3 and 4, respectively. The numbered peaks correspond to the identified volatile compounds listed in Table I. The BHT indicated in Figs. 2, 3, and 4 is thought to have been derived from the solvent and not as a naturally occuring substance although there might be a possibility that it comes from additives.

Basing on the profile of the chromatograms of each sample, acids appear to be the most abundant among the volatile compounds identified (Table I). The major acids in these fish sauces were found to be the same with those of Patis[3)] except in Nampla where isohexanoic acid could be included as a major one. Although quantitative analysis was not carried out in this study, chromatogram of Shottsuru (Fig. 2) shows that isopentanoic acid appears to be the most abundant followed by acetic, isobutanoic, *n*-butanoic and propionic acids. As in Nampla (Fig. 3), the order of acids based on their level of concentrations is similar to that of Shottsuru except that propanoic acid

TABLE I. VOLATILE COMPOUNDS IDENTIFIED IN SHOTTSURU, NAMPLA AND NOUCMAM

Peak No.	Compounds	Samples			References[r]	Methods of identification
		Shottsuru	Nampla	Noucmam		
Acids						
25	Acetic	+	+	+	a,b,c,d,e,f	GC-MS, t_R
28	Propanoic	+	+	+	a,c,d,e,f,g	GC-MS, t_R
30	Isobutanoic	+	+	+	a,c,d,e,f	GC-MS, t_R
31*	Pivalic	−	(+)	−	**	GC-MS
34	*n*-Butanoic	+	+	+	a,b,c,d,e,f,g	GC-MS, t_R
36	Isopentanoic	+	+	+	a,c,d,e,f,g	GC-MS, t_R
40	*n*-Pentanoic	+	+	(+)	a,f	GC-MS, t_R
41	4-Methyl valeric	+	−	−	**	GC-MS, t_R
47	Isohexanoic	+	+	(+)	a,f	GC-MS, t_R
49	*n*-Hexanoic	+	+	+	a,f	GC-MS, t_R
50	Phenylacetic	(+)	−	−	f	GC-MS, t_R
57	Isoheptanoic	−	(+)	+	a	GC-MS, t_R
59	*n*-Heptanoic	−	+	−	a	GC-MS, t_R
69*	3-Methoxy-4-hydroxybenzoic	−	(+)	−	**	GC-MS
70	3-Phenylpropionic	(+)	+	(+)	f	GC-MS, t_R
75	Benzoic	(+)	−	(+)	a,f	GC-MS, t_R
Alcohols						
1	Ethyleneglycol	(+)	(+)	(+)	**	GC-MS, t_R
6	Ethanol	+	+	+	a,e,f	GC-MS, t_R
15	Butan-1-ol	+	−	+	**	GC-MS, t_R
17	3-Methyl-1-butanol	−	(+)	(+)	f	GC-MS, t_R
24	Hexanol	(+)	−	+	**	GC-MS, t_R
26	Heptanol	−	(+)	−	a	GC-MS, t_R
44*	Divinyl carbinol	(+)	−	−	**	GC-MS
51	Benzyl alcohol	−	−	(+)	a	GC-MS, t_R
52	Phenyl ethanol	−	(+)	(+)	a	GC-MS, t_R
66*	3-Dehydro-diconiferyl alcohol	(+)	−	−	**	GC-MS
Nitrogen containing compounds						
20	2,5-Dimethyl pyrazine	(+)	−	(+)	a,f,g	GC-MS, t_R
21	2,6-Dimethyl pyrazine	(+)	−	(+)	a	GC-MS, t_R
23	2,3-Dimethyl pyrazine	(+)	−	(+)	**	GC-MS, t_R
65*	2, Methoxy-3-isopropyl pyrazine	−	−	(+)	**	GC-MS
71*	*n*-Formyl-5-methoxy-2-indolinol	−	(+)	−	a	GC-MS
72*	3-Methyl benzoquinoline	−	(+)	−	**	GC-MS
73*	Isonicotinamide	(+)	−	(+)	**	GC-MS
Sulfur containing compounds						
55*	1-Cyclohexy-3-phenyl thiourea	−	(+)	−	**	GC-MS
68*	2-*n*-Butyl-5-iso-butylthiophene	−	(+)	−	**	GC-MS
Lactones						
42	γ-Caprolactone	(+)	−	(+)	f	GC-MS, t_R
43*	4-Hydroxyhex-5-enoic acid γ-lactone	(+)	(+)	(+)	**	GC-MS
48*	Hex-2-enoic acid γ-lactone	(+)	(+)	(+)	**	GC-MS
Phenols						
63	Phenol	(+)	−	(+)	a	GC-MS, t_R
64	4-Ethyl phenol	−	−	(+)	**	GC-MS, t_R

Table 1. (continued)

Peak No.	Compounds	Samples			References[r]	Methods of identification
		Shottsuru	Nampla	Noucmam		
Carbonyls						
27	Benzaldehyde	+	−	−	f	GC-MS, t_R
15	Cyclopentanone	(+)	(+)	(+)	a	GC-MS, t_R
22	3-Hydroxy-3-methyl butan-2-one	(+)	(+)	(+)	**	GC-MS, t_R
52*	3-Hydroxy-1-methyl-anti-7-methyl-bicyclo heptan-2-one	−	(+)	−	**	GC-MS
67*	1,5-di-*t*-butyl-3,3-dimethyl-bicyclo-(3.1.0)hexan-2-one	(+)	(+)	(+)	**	GC-MS
Esters						
2	*n*-Butyl formate	(+)	(+)	−	**	GC-MS, t_R
3	Diethyl oxalate	(+)	−	(+)	**	GC-MS, t_R
4	Ethyl acetate	(+)	(+)	(+)	a	GC-MS, t_R
7	Ethyl propanoate	(+)	(+)	(+)	a	GC-MS, t_R
12	Ethyl *n*-butanoate	(+)	(+)	(+)	a	GC-MS, t_R
19	Ethyl lactate	(+)	−	(+)	**	GC-MS, t_R
60	Dibutyl phthalate	(+)	−	−	**	GC-MS, t_R
74*	Bis-(2-ethyl)hexyl phthalate	(+)	(+)	(+)	**	GC-MS
Hydrocarbons						
5	Decane	(+)	(+)	(+)	**	GC-MS, t_R
8	Dodecane	(+)	(+)	(+)	**	GC-MS, t_R
9*	3-Methyl heptane	(+)	(+)	(+)	**	GC-MS
10*	2,2,3-Trimethyl pentane	(+)	(+)	(+)	**	GC-MS
11*	2,2,4-Trimethyl heptane	(+)	(+)	(+)	**	GC-MS
13*	4-*n*-Methyl nonane	(+)	(+)	(+)	**	GC-MS
18	*cis*-3-Pentene	(+)	(+)	(+)	**	GC-MS, t_R
14*	5-*n*-Butyl nonane	(+)	(+)	(+)	**	GC-MS
37*	2,6,10-Trimethyl tridecane	(+)	(+)	(+)	a	GC-MS
38*	2,6,10-Trimethyl hexadecane	(+)	(+)	(+)	a	GC-MS
39*	2,6,10-Trimethyl pentadecane	(+)	(+)	(+)	a	GC-MS

* Tentative identification.
** Not previously reported.
t_R, retention time.
+, detected; −, not detected; (+), newly detected in the three samples.
r: a, Sanceda *et al.* (1984); b, Truong Van Chom (1957); c, Dougan and Howard (1975); d, Beddows *et al.* (1979); e, Nonaka *et al.* (1975); f, McIver *et al.* (1982); g, Ide *et al.* (1982).

seems to be higher than isobutanoic acid in the former. Noucmam gave a somewhat different pattern when compared with the other two. Acetic acid seems to be the highest in this sample. These results show different patterns with those reported by McIver *et al.* for Nampla[6] and Nonaka *et al.* for Shottsuru and Noucmam.[7] As we mentioned before, these differences might be due to the different methods used in the analyses. However, comparison of the result on analysis of Patis[3] with these three samples also gave a different result inspite of the fact that the same procedure of analysis was used.

In order to examine the role of volatile acids in the differences of aroma of these samples, we prepared mixtures of acids using the authentic ones with similar proportions of concentrations of major acids found in the samples including those of Patis. The odor of each mixture was observed and was compared to the odor of the whole as well as the acidic fraction of each sample. It was noticed that the same acid note could be felt in the standard mixture but each differ in their characteristics. For instance, the mixture with a highest con-

centration of n-butanoic acid (as in the Patis sample) gave a very disagreeable odor and the n-butanoic acid smell was very much apparent whereas in the mixtures where isopentanoic acid was most abundant (as in the Shottsuru and Nampla samples) did not give such a strong disagreeable odor but rather a milder one although in this case the butanoic smell could still be felt. Isopentanoic acid has that butanoic acid like smell but was not unpleasant.

The acidic fractions of each sample including that of Patis exhibited similar acid smells but differed in the degree of intensity. Comparing the respective standard acid mixtures with the acidic fraction of each sample, it was observed that in the acidic fraction of Patis, n-butanoic acid which was found to be the most abundant, did not exhibit its strong characteristic smell compared to the standard mixture prepared with the same proportion of acid concentration. In Shottsuru, the isopentanoic acid smell was hardly distinguished unlike in the standard mixture prepared with this concentration. The same tendencies were observed in the acidic fractions of Nampla and Noucmam and the minor acids were thought to have a milder effect on it.

In our previous study,[2] a reconstitution test was done by combining different fractions of Patis distillate and it was found that there were varying differences in smell in the different mixtures. Comparison of the authentic acid mixtures with the whole of each sample showed a very different odor although the presence of the acid smell could not be ignored. Strong characteristics of individual acids could hardly be distinguished. This might suggest the influence of the minor volatile compounds present in other fractions.

Noucmam contains a few more alcohols compared with the other two (Table I). Ethanol was found in all the samples at a fair concentration. Phenyl ethanol found in the two but not in Shottsuru was also found at a fair concentration. Nitrogen-containing compounds were found to be more in Shottsuru and Noucmam but hardly in Nampla. Pyrazines were not detected in Nampla as shown in Table I, or their concentrations were too low to be detected. This supports the subjective observation that Nampla has a milder burnt smell than the other two samples. Noucmam was found to have a very burnt smell and this may be attributed to the presence of pyrazines. Some of the nitrogen containing compounds in this analysis were only tentatively identified by mass spectra. 2,5-Dimethyl pyrazine was previously reported to be present in Nampla[6] but not the rest of the nitrogen-containing compounds. Pyrazines are heterocyclic compounds and have been characterized to contribute to the unique flavor and aroma associated with the roasting or toasting of numerous foods. In this study, the same lactones found in Nampla were reportedly present in the study of Thai fish sauce by McIver *et al.*[6] but not in the study of Nonaka *et al.*[7] on Shottsuru and Noucmam. Lactones which were found in all the samples in this study might play a role in the sweet aroma of these fish sauces. Two sulfur-containing compounds were detected in Nampla but not in Shottsuru and Noucmam. Benzaldehyde was the only aldehyde identified in Shottsuru but was not found in the other two samples (Table I).

A considerable number of mass spectra of these samples revealed characteristic, fragment patterns of hydrocarbons but a few of them were only tentatively identified as shown in Table I. Aside from those tentatively identified, compounds corresponding to peaks No. 29, 32, 33, 35, 45 and 46, were considered normal hydrocarbons and those peaks with long retention times were also deduced to be normal or branched bydrocarbons with high molecular weights. In general, normal hydrocarbons have a very weak smell so that they could hardly contribute to the aroma of fish sauce.

There were two phenolic compounds detected in Noucmam, and phenol appeared to be in high concentration. In the two other samples, only phenol was found but did not have as high concentration as the former sample.

Although the data was not published, analysis of our model experiment fish sauce which were not treated with any additives revealed the presence of phenol and it is believed that this compound occurs naturally in the sample and is also thought to be a natural substance in the three samples analyzed above, although of varied concentrations and except with Noucmam which contained an unexpectedly large amount which we can not explain.

The differences in aroma observed in the analyzed samples have been thought to be due to the differences in the level of concentrations of the major acids. Moreover, some differences in the kinds of the minor volatile compounds were observed and these could also possibly contribute to the differences of aroma in the samples. As was observed in the analysis on the aroma of Patis,[2] although *n*-butanoic was considered the most abundant acid in the sample, its impact on the overall aroma of sauce was not well defined when blended with the other volatile compounds. Minor volatile compounds like pyrazines could contribute to the burnt flavor, lactones may have an influence on the sweet aroma, sulfur containing compounds may have an effect on the disagreeable odor and other minor compounds may possibly contribute to the differences of aroma in the samples.

Since these samples were commercially purchased, we have limited information as to their production conditions as well as the species of fishes used and so their influence on the total aroma is not clear, however, results of the study showed that there were differences in the total aroma of the commonly used and representative samples of fish sauces from different countries and these differences were attributed to the aroma characteristics of the major as well as the minor volatile compounds. A total of 50, 44 and 49 volatile compounds were identified in Shottsuru, Nampla and Noucmam respectively, and out of these, a total of 37, 31 and 38 volatile compounds correspondingly were newly identified.

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